

ALS Protein Crystallography Reveals How Pieces Fit Together

After decades of effort, techniques for crystallizing ribosomes and analyzing their structures have begun to yield rich dividends. Within the space of a month, several research groups recently reported on the solution of ribosome structures. Among these, the results obtained at the ALS by Harry Noller and his team from the University of California, Santa Cruz, stand apart. While other groups focused on the component parts (subunits) of the ribosome, Noller's group looked at the overall ribosome and how its parts fit together. About 2.5 million times more massive than a hydrogen atom, these ribosomes are the largest asymmetric structures solved by crystallography to date.

Ribosomes are the cell's protein manufacturing plants. In the ribosome, raw materials in the form of amino acids are combined according to blueprints provided by ribonucleic acid (RNA) to produce all the proteins necessary for life. To perform this complex task,

the ribosome has evolved a complex structure, the rough outlines of which are already known. Two subunits, one larger (50S) and one smaller (30S), together constitute the whole (70S) ribosome. The interface between the subunits contains several cavities where proteins (chains of amino acids) are assembled.

The order of the amino acids in the chain is determined by the sequence of nucleotides in a strand of messenger RNA (mRNA), which moves through the ribosome cavity like a conveyor belt. Each combination of three nucleotides (codon) in the mRNA strand forms base pairs with the complementary nucleotides (anticodon) in molecules of transfer RNA (tRNA). Each tRNA molecule carries a specific amino acid. Bonds are formed between adjacent amino acids and the growing chain exits the ribosome through a tunnel.

Bacterial ribosomes, which have the same basic structure as those of all life forms, are smaller than

others and are therefore the most studied. Noller's team successfully crystallized 70S ribosome complexes (ribosomes plus various mRNA and tRNA fragments) from the bacterium *Thermus thermophilus*. Taking advantage of the high photon flux and collimation of Beamline 5.0.2 at the ALS, the researchers used multiple-wavelength anomalous diffraction (MAD) techniques to obtain electron-density maps of the ribosome complexes with a resolution as good as 7.8 Å.

In addition to confirming features of ribosome structure already known through other types of studies, the electron-density maps reveal many interesting new details. For example, the images indicate how tRNAs are bound to various sites in the ribosome. At the site where codon-anticodon matches are recognized, only weak contacts between the ribosome and tRNA were observed, suggesting a degree of flexibility. In contrast, at the site where the amino acid chain begins to form, the ri-

bosome rigidly grips the tRNA with six "fingers" of electron density, stabilizing and orienting both tRNA and mRNA components. Another striking feature seen in the images is an RNA helix that runs along the length of the 30S subunit. This single feature contributes about half of all the contacts between the two subunits and may function as a relay switch, linking events occurring in the two subunits by alternating between two different configurations.

These results reinforce the impression that the ribosome is a dynamic molecular machine with moving parts and a very complicated mechanism of action. Through these studies, Noller et al. are, in a sense, reverse-engineering the ribosome: attempting to understand its function by examining how it is constructed. Toward this end, Noller and his colleagues are already working on improving their resolution and obtaining images of ribosomes at different stages of protein synthesis.

H.F. Noller (408-459-2453), Biology Department, University of California, Santa Cruz.

J.H. Cate, M.M. Yusupov, G.Zh. Yusupova, T.N. Earnest, H.F. Noller, "X-ray Crystal Structures of 70S Ribosome Functional Complexes," *Science* **285** (1999) 2095.

RESEARCH FUNDING: National Institutes of Health; Agouron Institute. Operation of the ALS is supported by the Materials Science Division of the U. S. Department of Energy.



SOLVING THE RIBOSOME PUZZLE

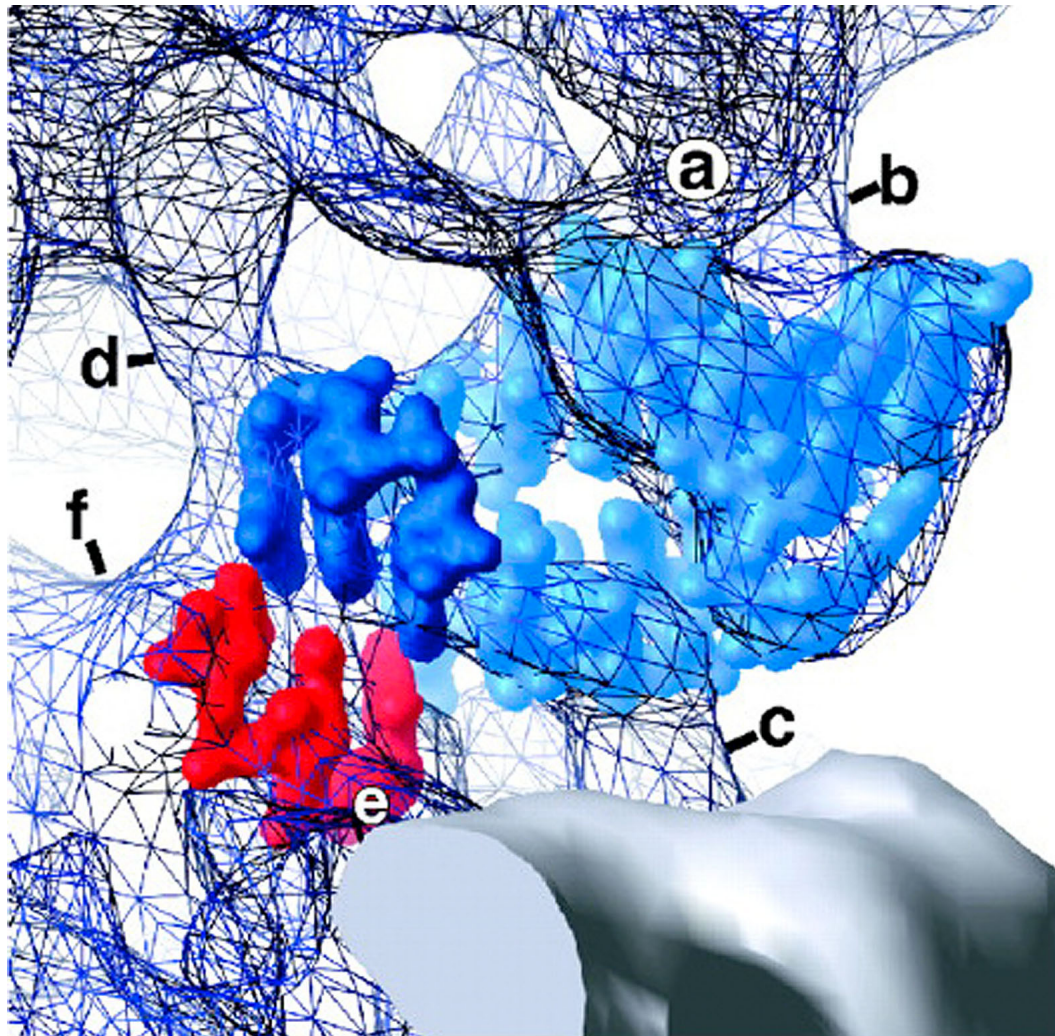


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- **Ribosomes synthesize protein in all living organisms**
 - *Structural knowledge —> More effective antibiotics, insights into origin of life*
 - *Asymmetric molecule, 2.5 million times more massive than a hydrogen atom*
 - *Other crystallography studies focused on ribosome subunits*
- **Crystallography of whole *T. thermophilus* ribosome complexes at ALS**
 - *Largest asymmetric structures solved to date, 7.8-Å resolution*
 - *High flux, collimation of light from ALS facility crucial to success*
- **Observations of interactions between ribosome components**
 - *Confirm structures already known through other techniques*
 - *Reveal new structural features*
- **Future directions**
 - *Improve resolution to obtain detailed atomic structure of ribosome*
 - *Obtain structures at different stages of protein synthesis;*

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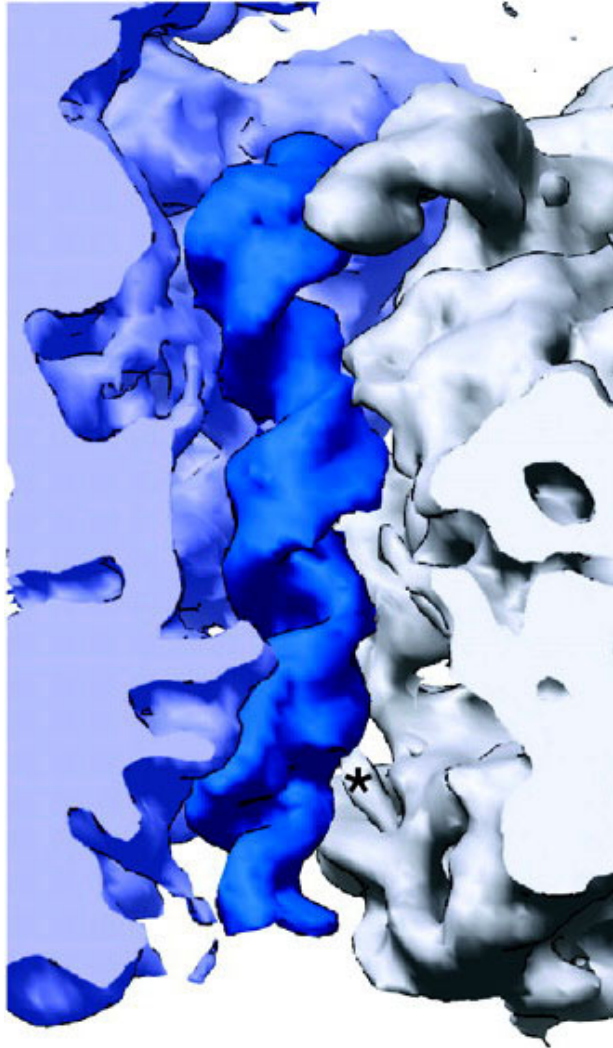


Six “fingers” of ribosomal electron density (labeled a-f) tightly grip mRNA (red) and tRNA (blue) components to facilitate protein synthesis.

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*RNA helix (blue) packed
into interface between the
two ribosomal subunits
(purple and gray).*



*This feature may relay
information from one
subunit to the other*